

THE REPRODUCTION AND EARLY LIFE HISTORIES OF THE
GASTROPODS *NOTOACMAEA PETTERDI* (TEN.-WOODS),
CHIAZACMAEA FLAMMEA (QUOY AND GAIMARD) AND
PATELLOIDA ALTICOSTATA (ANGAS) (FAM. ACMAEIDAE)

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Synopsis

Notoacmaea petterdi, with an externally fertilized egg 150μ in diameter, develops into a planktonic lecithotrophic trochophore in 16 hours, remains planktonic for 30 hours, during which it transforms into a simple veliger, then settles, alternating crawling with intermittent swimming, during a period of eight days. Feeding does not begin until settlement is permanent, by which time metamorphosis is well advanced. Development in *Chiazacmaea flammea*, with a 130μ egg, and *Patelloida alticostata*, with a 150μ egg, follows a similar course, but in *C. flammea* permanent swimming is maintained for 60 hours and permanent settlement attained after four more days, while in *P. alticostata* swimming for 60 hours is followed by settlement over three more days. The form and dimensions of the eggs and larvae in these three species resemble those of *Acmaea testudinalis*, but associated with a more prolonged subsistence on yolk, *C. flammea*, *P. alticostata* and especially *N. petterdi* have a more extended swimming-distributive phase than *A. testudinalis*.

INTRODUCTION

Although several species of acmaeid limpet are commonly represented along the New South Wales coast (Dakin, 1953), their reproduction and early life histories have not been investigated (Anderson, 1960). Little is known of larval development in the Acmaeidae, the only comprehensive description being that of Kessel (1964) for *Acmaea testudinalis*. The present study of *Notoacmaea petterdi*, *Chiazacmaea flammea* and *Patelloida alticostata* shows that their larval development differs from that of *A. testudinalis* in a number of interesting ways.

MATERIALS AND METHODS

For *N. petterdi*, which inhabits upper littoral vertical rock faces exposed to the ocean surf, animals collected from the rock platform at Harbord, N.S.W., in July and August 1964 and in January 1965 were found to contain ripe gametes at both periods, suggesting that breeding occurs throughout the year. For *Chiazacmaea flammea*, which lives in association with oysters intertidally in estuarine waters, animals collected from the shores of Middle Harbour, N.S.W., in August 1964 and January 1965 also contained ripe gametes at both times, similarly indicating a prolonged breeding season. For *Patelloida alticostata*, which lives at very low levels on intertidal coastal rock platforms, animals containing ripe gametes were obtained from Long Reef, N.S.W., in January 1965, but have not yet been examined at other times of the year.

Larvae of each species were obtained by artificial fertilization, after releasing eggs and sperm by dissection of the adults. Eggs were divided into batches of about 100 and allowed to stand in 300 ml. of filtered sea-water for 30 minutes before adding a few drops of sperm suspension. Swimming trochophores resulting from successful fertilization were transferred by means of a pipette to Petri dishes of filtered sea-water, a similar transfer to fresh sea-water being effected each day until permanent settlement had occurred. All cultures were maintained at 20°C.

RESULTS

Notoacmaea petterdi

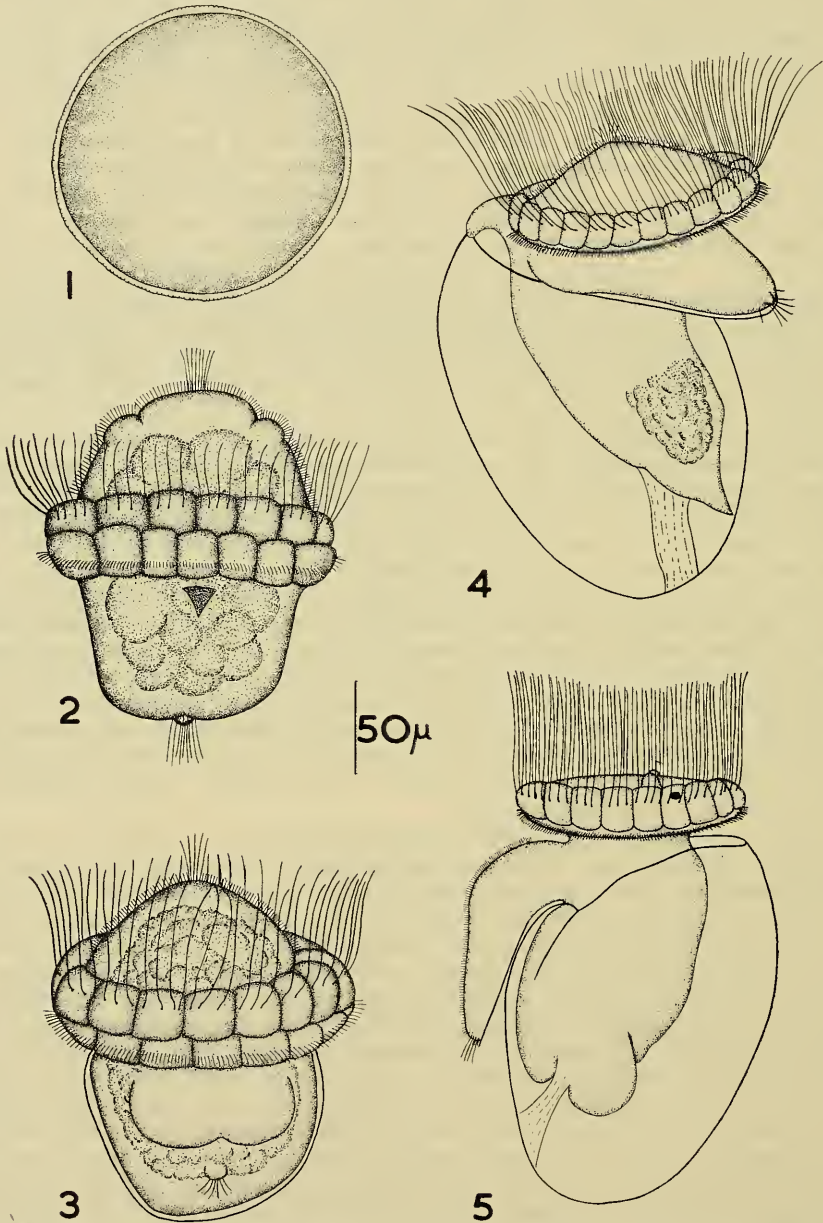
The mature oocytes of *N. petterdi* (Fig. 1) are pink in colour, uniformly yolky and opaque, covered by a thin egg membrane and, after immersion in sea-water for 30 minutes, spherical, with a diameter of 150μ . Sea-water immersion also causes a thin layer of colourless jelly to swell up on the surface of the egg, but there is no indication of adhesion between eggs, suggesting that they float freely in the water following natural spawning. Sixteen hours after fertilization, free-swimming yolky trochophores (Fig. 2) are found swimming near the water surface by means of a prototroch of long coarse cilia. The action of the prototroch is intermittent, the larva either swimming at random in short darting bursts or drifting with the prototroch folded forwards. The prototrochal ciliary beat has a clockwise metachronal rhythm but the larva itself does not rotate while swimming. Behind the prototrochal ring is a ring of short vibrating cilia, while anteriorly and posteriorly lie apical and terminal tufts of stationary cilia, held extended while the larva is in motion. The episphere with its paired antero-lateral protuberances is also covered by short, slowly waving cilia. Due to the internal mass of yolky macromeres, the trochophore retains the pink coloration of the egg, but its outer parts are colourless.

At about 19 hours, the foot rudiment begins to grow out ventrally behind the prototroch and during the next five hours the trochophore develops into a simple early veliger (Fig. 3). The prototroch shows little change of form, but its action becomes more vigorous and continuous, so that long periods of steady swimming in a semi-upright position near the water surface are interspersed with short resting periods during which the larva gradually sinks through the water. No unidirectional response to light is observed at this or any other stage of development. The episphere in the early veliger is already reduced in size, and has lost its paired protuberances, although its apical tuft and general ciliation are retained. The hyposphere, in contrast, is enlarged and elaborated, with a conspicuous, bilobed, ventral foot rudiment and a globular, colourless, dorsal shell. Due to dorsal enlargement of the hyposphere, the original terminal tuft of cilia is pushed postero-ventrally and now sprouts from a small protuberance behind the foot. The interior of the veliger is still occupied by a pinkish mass of yolky macromeres.

During the second day of development, the veliger continues to swim in an upright position near the water surface, with the velar cilia maintaining their clockwise metachronal beat, rising through the water, then sinking again at intervals when the velar cilia come to rest. At the same time, the veliger (Fig. 4) increases greatly in size and shows numerous structural changes. In the velum, the velar cilia grow longer and retain their vigorous activity, but the velar cells become much smaller, indicating the onset of a gradual metamorphosis. The episphere, although retaining its ciliation, also becomes smaller, being much flatter at the end of the second day than at the beginning. The foot, in contrast, increases in size as a triangular wedge incorporating the terminal tuft and its protuberance at the apex, and secretes an operculum on its posterior face. The colourless larval shell is greatly enlarged, with the visceral mass, attached posteriorly to the shell by paired columella muscles, occupying only part of it, the remainder being occupied by the mantle cavity. Torsion occurs during the second day, so that the mantle cavity becomes dorsal in position, but neither withdrawal into the shell nor muscular movements of the animal are observed during this time. The main mass of yolk is now concentrated in the visceral mass, but the remainder of the tissues are also semi-opaque and not obviously differentiated.

Further progress in development and metamorphosis during the third day (Fig. 5) is accompanied by a change in behaviour. From continuous swimming, the behaviour of the larva alters to long periods of sedentary attach-

ment to the bottom interspersed with brief slow swimming excursions upwards through the water. Even agitation of the dish in which the larvae are maintained fails to alter this pattern. The larval shell grows no larger, but the velum becomes slightly smaller and its cilia begin to shrink. The episphere



Figs 1-5. *Notoacmaea petterdi*. 1. Mature oocyte; 2. trochophore, 16 hr., ventral view; 3, veliger, 24 hr., ventral view; 4, veliger, 41 hr., ventrolateral view; 5, veliger, 65 hr., lateral view.

becomes flattened and shows the onset of differentiation of the eyes as a pair of dark brown dorso-lateral pigment spots and tentacles as a pair of blunt, short protuberances ventral and median to the eyespots. The head and visceral mass retain the pinkish-brown opacity indicative of continued lecithotrophy,

but the beginnings of differentiation of the gut can be discerned in the visceral mass and the columella muscles become contractile, producing complete withdrawal into the shell in response to stimulation. The foot also becomes highly muscular and mobile, elongates slightly in a posterior direction, and develops a layer of short, continuously-beating cilia over its ventral surface. Slow creeping in an exploratory manner over the substratum on the ciliated sole of the foot begins towards the end of the third day, but the larva is unstable in the creeping position and frequently tips over to one side or the other. Spasmodic beating of the velar cilia during creeping appears to assist in maintaining balance while the foot is in this rudimentary condition.

During the fourth day, although brief swimming excursions continue, metamorphosis becomes more evident and the capacity for strong attachment and creeping in a straight line on the foot is enhanced (Fig. 6). The velum continues to shrink, its cilia becoming finer and shorter, while the tentacles elongate and become highly mobile. The ciliated foot is also greatly elongated antero-posteriorly, while in the visceral mass, although some yolk remains, the coil of the intestine leading to the anus becomes conspicuous. Crawling on the foot occurs in the typical snail manner, the shell being held upright and the tentacles extended forwards, outwards and downwards, rhythmically tapping the substratum in front of the animal. In contrast to their activity during the earlier phase of more unstable crawling, the velar cilia now remain at rest, partly covered by the shell, as the animal creeps along. While attached by the sole of the foot, however, the animal cannot withdraw fully into the shell, part of the foot remaining uncovered when columella muscle contraction occurs and the shell is clamped down on the body. Full withdrawal is possible only if the foot becomes detached from the substratum.

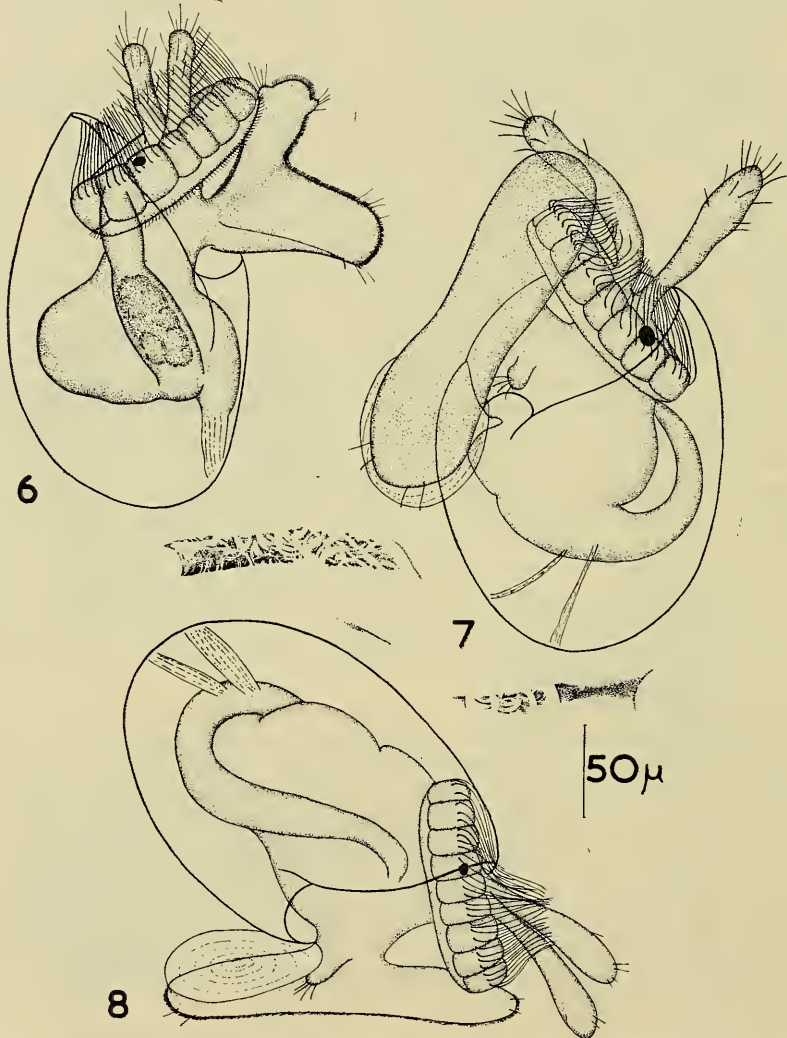
During the fifth day, the same type of crawling behaviour is pursued more vigorously, with the tentacles and the anterior end of the foot pushing out from side to side in what appears to be an exploratory manner, and with frequent changes of direction. At the same time, internal differentiation proceeds rapidly in the visceral mass, the coiled gut becoming more obvious, and the tentacles grow longer and the eyespots larger. A general increase in muscular activity is also evident during this time. The velum, however, does not appear to undergo further reduction.

Progressively, development in the same direction continues during the next four days, growth of the foot and tentacles being accompanied by further differentiation of the visceral mass (Fig. 7). The velum reduces in size only very slowly during this time, although its cilia become finer and shorter and the swimming excursions made become more and more infrequent and more and more feeble. On the last of these days, further addition to the margin of the shell begins, giving it the circular marginal outline of an incipient adult shell, and following this, on the tenth day, the capacity for swimming is lost. The animal (Fig. 8) crawls actively on its foot, and if dislodged, immediately reattaches. The gut is by now very well developed and although feeding has not yet begun, it is obvious that it must soon do so. Development was not followed beyond this point.

Chiazacmaea flammea

The mature oocytes of *C. flammea* (Fig. 9) are brown in colour, uniformly yolky and opaque, covered by a thin egg membrane and, after immersion in sea-water for 30 minutes, spherical, with a diameter of 130μ . A layer of colourless jelly covering the egg swells to a thickness of about 10μ , but there is no evidence of adhesion between eggs. Like those of *N. petterdi*, they probably float singly and demersally after natural spawning. Fourteen hours after fertilization, free swimming trochophores (Fig. 10) are found near the water surface, swimming by a metachronal clockwise beating of the long thick cilia of the prototroch, short curving bursts being interspersed with periods of rest.

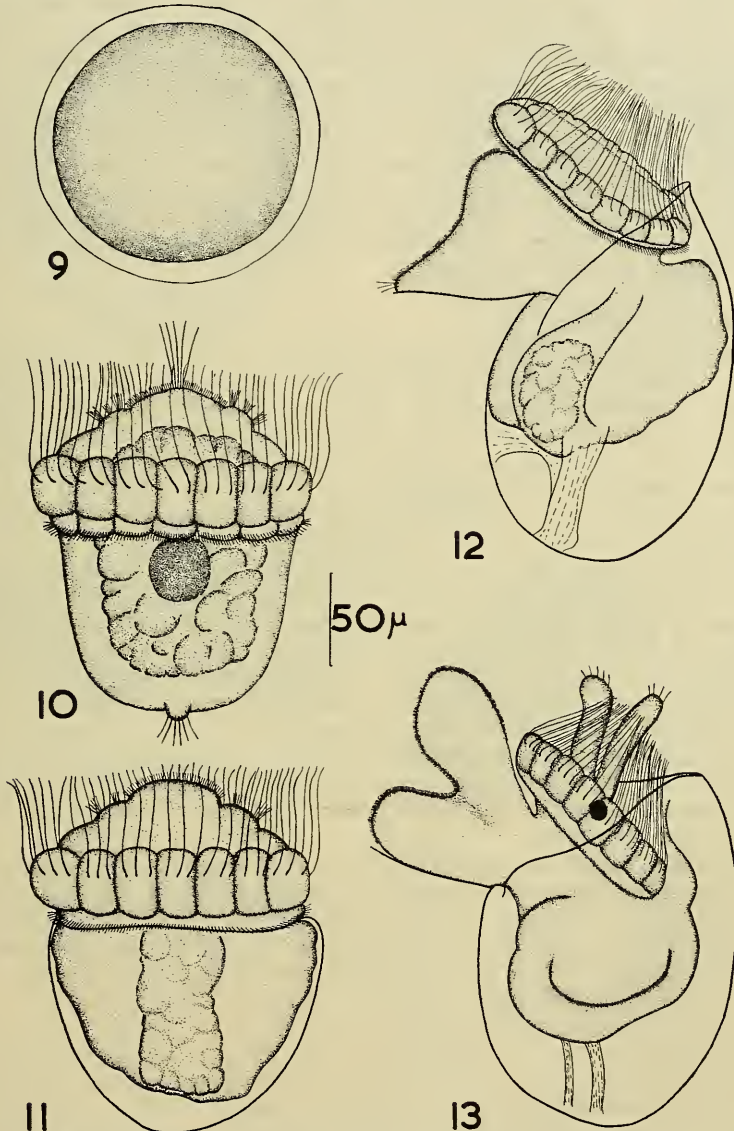
The brown coloration of the egg is retained in the yolky interior of the trochophore, occupied by macromeres. On the conical episphere, in addition to a general motionless ciliation and paired antero-lateral protuberances, is an apical tuft of long cilia, normally held stiffly upright during swimming but also showing slow bending and waving movements. The hyposphere is unciliated save for a terminal tuft of long motionless cilia borne on a small postero-ventral protuberance. In all general respects the trochophore of *C. flammea* is similar to that of *N. petterdi*.



Figs 6-8. *Notoacmaea petterdi*. 6, Early metamorphosis, 90 hr., lateral view; 7, continuing metamorphosis, 6½ days, ventral view; 8, first permanently settled stage, 10 days, lateral view.

Development proceeds rapidly. Within the next five hours (Fig. 11), the hyposphere enlarges dorsally, the globular larval shell is secreted, and a small simple foot rudiment grows out mid-ventrally behind the prototroch. The latter undergoes little change, but swimming in a semi-upright position becomes more or less continuous, with periods of slow swimming interspersed with short faster curving bursts. The episphere shows no change other than loss of the apical tuft.

During the remainder of the first and throughout the second day (Fig. 12), steady swimming near the water surface continues as the veliger becomes progressively elaborated. In spite of this, the prototroch undergoes reduction in size during this time, although its cilia remain long and active. The episphere becomes flattened and unciliated, while behind the prototroch, the foot rudiment



Figs 9-13. *Chiazacmaea flammea*. 9, Mature oocyte; 10, trochophore, 14 hr., ventral view; 11, veliger, 19 hr., dorsal view; 12, veliger, 44 hr., lateral view; 13, early metamorphosis, 4½ days, lateral view.

enlarges, growing posteriorly, secretes an operculum, and becomes ciliated over its ventral surface. The larval shell is greatly enlarged and the visceral mass undergoes torsion and begins to show differentiation of the gut. Some muscular activity also becomes evident in the foot and visceral mass, but withdrawal into the shell does not occur. In swimming, the velum is projected antero-dorsally, with the visceral mass and shell suspended below it, and the

beating of the velar cilia draws the animal along in a vertical position with the foot trailing. The visceral mass is still opaque, due to the presence of brownish yolk reserves.

Steady swimming and progressive development continue during the third day, the visceral mass becoming more differentiated and the foot longer and more muscular. During the fourth day (Fig. 13), paired dark brown eyespots are developed dorso-laterally on the episphere, while ventral and median to them paired tentacles grow out. At the same time, the velum begins to shrink and swimming becomes interspersed with periods during which the larva settles and crawls very slowly and feebly on its foot, with the shell held upright. Gradually over the next three days, with little further change in appearance, the capacity for swimming is lost and crawling greatly improved. The mode of crawling is similar to that of *N. petterdi* at the corresponding stage. By this time, most of the yolk reserves have been utilized but there is no evidence that feeding begins before settlement has become permanent. Development was not followed beyond this point.

Patelloida alticostata

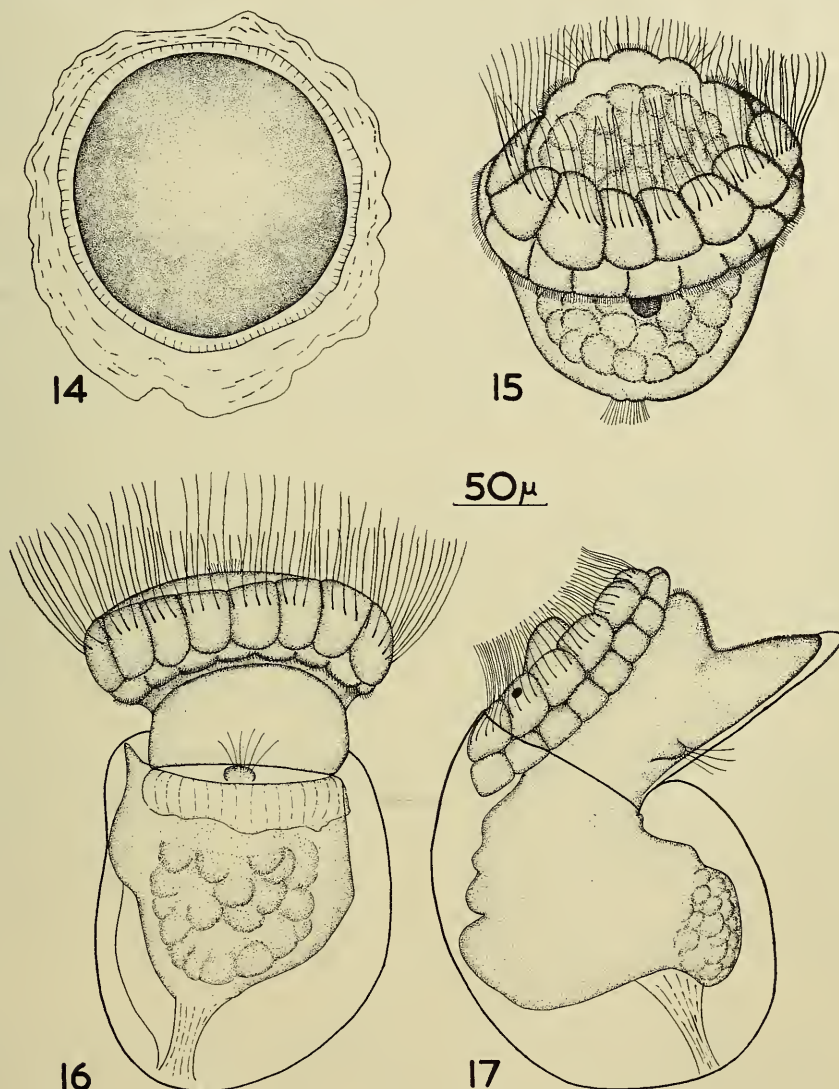
The mature oocytes of *P. alticostata* (Fig. 14) are yellow in colour, uniformly yolky and opaque, covered by a thin egg membrane and, after immersion for 30 minutes in sea-water, spherical, with a diameter of 150 μ . Sea-water immersion also causes a double layer of colourless jelly to swell up around the egg, a uniform, dense, inner layer being covered by a less dense, irregular, outer layer. Some tendency to adhesion is observed between eggs, and it is possible that in natural spawning the eggs adhere temporarily as a gelatinous egg mass until hatching and escape of the trochophores occurs.

Trochophores (Fig. 15) are found swimming actively near the surface of the water 18 hours after fertilization. They are semi-opaque, filled internally with a mass of yellow yolky macromeres, and differ from the trochophores of *N. petterdi* and *C. flammea* in a number of ways. The prototroch is more protuberant, with a large number of much finer cilia and, although these beat in clockwise metachronal rhythm in the usual way, the swimming of the trochophore is a slow continuous straight line progression, without the darting, curving movements characteristic of the other species. The conical episphere is finely ciliated, with low paired antero-lateral protuberances bearing long cilia, and with little development of an apical tuft. The hyposphere bears the usual terminal tuft of fine cilia.

During the second day of development (Fig. 16), the prototroch enlarges slightly as a velum, its cilia become more powerful and steady swimming near the water surface continues. At the same time, the episphere flattens and loses much of its ciliation, while the hyposphere enlarges and differentiates as a dorsal visceral mass secreting a globular shell and a ventral foot rudiment secreting a posterior operculum. The visceral mass is still very yolky. Torsion occurs towards the end of the second day.

During the third day of development (Fig. 17), steady swimming in a semi-upright position continues, but the velum begins to shrink. The episphere also becomes more flattened and develops a pair of brown eyespots. Little change occurs in the foot rudiment other than development of a fine ventral ciliation, but the larval shell becomes further enlarged and the visceral mass further differentiated, showing retention of yellow yolk reserves mainly ventrally. Muscular movements in general are not conspicuous in the larva at this stage, and, although complete withdrawal into the shell is possible, accompanied by sinking through the water, there is no attachment or creeping on the substratum. The early phases of metamorphosis, however, proceed rapidly during the fourth day, with further reduction of the velum, outgrowth of paired tentacles on the head, elongation of the foot, and further differentiation of the organs of the visceral mass. Muscularity is greatly increased, and by the

end of the fourth day crawling predominates over swimming. After two further days, although the reduced velum is still retained, the crawling habit has become permanent and the young animal is closely similar to the young of *N. petterdi* illustrated in Figure 8. Feeding does not begin until settlement is complete.



Figs 14-17. *Patelloida alticostata*. 14, Mature oocyte; 15, trochophore, 18 hr., anterioventral view; 16, veliger, 42 hr., ventral view; 17, veliger just entering metamorphosis, 66 hr., lateral view.

DISCUSSION

In *N. petterdi* and *C. flammea*, eggs are probably spawned singly into the water, as in *Acmaea virginea* and *Acmaea fragilis* (Boutan, 1898; Willcox, 1898, 1900), since they show no tendency to adhere after artificial release. In *P. alticostata*, in contrast, released eggs adhere temporarily by their outer jelly coats, and it is possible that in natural spawning they aggregate as a transient egg mass, as in *Acmaea testudinalis* (Kessel, 1964).

For *Acmaea testudinalis*, Kessel (1964) has shown that at 12°C, the 140 μ egg hatches as a free-swimming trochophore in 10–13 hours. It remains lecithotrophic for about 50 hours, attaining during this period a well developed pretorsional veliger stage with a circular monotrochal velum. Planktotrophy then begins, torsion occurs and the veliger remains planktotrophic, with further development of the shell, foot and visceral mass, for about 25 hours. Towards the end of this period, eyes and tentacle rudiments begin to differentiate in the head and swimming begins to alternate with periods of crawling on the now well developed foot. Permanent settlement, with crawling and feeding, is established within 15 hours (i.e., by the time the larva is four days old) but metamorphosis, with loss of the velum and operculum, further elaboration of the head and foot and onset of secretion of the adult shell, does not become obvious until 11 days after settlement.

Development in *N. petterdi*, *C. flammea* and *P. alticostata*, while generally similar in the three species, differs from that of *Acmaea testudinalis* in a number of ways. Although egg dimensions, mode of spawning and fertilization, and early hatching as a lecithotrophic free-swimming trochophore are shared in common, and planktonic life is equally brief (about 30 hours in *N. petterdi* and 60 hours in *C. flammea* and *P. alticostata* at 20°C, compared with about 75 hours in *Acmaea testudinalis* at 12°C), lecithotrophy is maintained throughout planktonic life. Development of the eyes and tentacles and onset of velar shrinkage are more precocious than in the planktotrophic larva of *A. testudinalis*. At the same time, the transition to permanent settlement, preceding the onset of feeding, occurs more slowly, taking three days in *P. alticostata*, four days in *C. flammea* and eight days in *N. petterdi*, and is accompanied by gradual metamorphosis and functional differentiation of the organs of the visceral mass.

Thus *N. petterdi*, *C. flammea* and *P. alticostata* are adapted to a more economical utilization of yolk reserves than *Acmaea testudinalis*. The biochemical basis of this difference is obscure, but if we regard the planktotrophic development of *A. testudinalis* as primitive, it is a difference which appears to offer certain advantages. In the absence of velar elaboration, planktotrophic life in *A. testudinalis* is necessarily brief and rapid permanent settlement is essential to the transition from planktonic to bottom feeding, even though the onset of metamorphosis is delayed for several days. In *C. flammea*, *P. alticostata* and especially *N. petterdi*, with similar larval dimensions and a similar simple velum, permanent planktonic life is equally brief, but planktonic feeding is obviated and the delayed onset of bottom feeding is associated with intermittent swimming excursions during the several days before secretion of the adult shell begins. Thus development is more direct but the distributive planktonic phase is more prolonged. From such a mode of development it is but a short step to the ovoviviparity and birth as a crawling juvenile described for the Arctic *Acmaea rubella* by Thorson (1935).

Acknowledgements

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